

# Physical Properties of Membrane Lipids: Biological Relevance and Regulation

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Introduction .....	232
Pertinent Properties of the Cell Envelope .....	233
<i>E. coli</i> Fatty Acid Auxotrophs .....	233
Specific inhibitors of Fatty Acid Synthesis .....	234
LIPID BILAYER PHASE PROPERTIES .....	234
Empirical Rules Deduced from Model Systems .....	234
Physical Properties of the <i>E. coli</i> Membrane Lipids .....	236
Phase transitions .....	236
(i) X-ray diffraction .....	236
(ii) DSC .....	237
(iii) Fluorescent probes .....	237
(iv) Spin labeling .....	237
Phase separations. (i) Physical studies .....	237
(ii) Freeze-fracture electron microscopy .....	239
Lateral movement of lipids .....	240
CORRELATIONS BETWEEN MEMBRANE-ASSOCIATED PHYSIOLOGY AND LIPID PHYSICAL PROPERTIES .....	240
General Physiology .....	240
Lactose Transport System .....	240
Relevant properties of lactose transport system .....	240
Transport system induction and lipid synthesis .....	241
Transport and lipid phase changes .....	241
Other Transport Systems .....	243
Sugar transport systems .....	243
Amino acid transport systems .....	243
Other Physiological Processes .....	243
Lipid requirement for alkaline phosphatase derepression .....	243
Chemotaxis .....	244
Initiation of DNA synthesis .....	244
Methylgalactoside permease induction .....	244
Cell integrity .....	244
Enzymatic activity .....	244
Requirement for Lipids with Specific Physical Properties .....	245
Requirement for membrane liquidity .....	245
Minimum UFA content .....	246
Minimum saturated fatty acid content .....	246
Significance of temperature-induced alterations in fatty acids .....	247
REGULATION OF LIPID PHYSICAL PROPERTIES .....	247
Possible Lipid Alterations Producing Altered Phase Transitions .....	247
Sites of Control .....	247
In Vitro Studies .....	247
(i) Acyltransferase specificity .....	248
(ii) Fatty acid synthetase .....	249
In Vivo Studies .....	249
(i) Determination of fatty acid chain length .....	249
(ii) Regulation of the saturated-to-unsaturated fatty acid ratio .....	250
(iii) Temperature control of fatty acid composition .....	250
CONCLUSION .....	251
LITERATURE CITED .....	252

## INTRODUCTION

The physical properties of the phospholipids found in biological membranes have been the subject of a large volume of recent work. The

touchstones of this work were probably the demonstrations (24, 110) of lipid phase transitions in *Acholeplasma* (formerly *Mycoplasma*) *laidlawii*. This organism has been the subject of

many studies because it is quite easy to manipulate the fatty acid content of its membrane phospholipids. However, relatively little is known about the membrane physiology of *A. laidlawii*. In contrast, a wealth of information is available concerning the membrane-associated functions of *Escherichia coli*. The isolation of *E. coli* mutants in membrane lipid synthesis (102) therefore has resulted in *E. coli* largely supplanting *A. laidlawii* in studies relating lipid physical properties to physiological processes.

This article will concentrate on a critical evaluation of the literature relating to *E. coli*. To provide a background, we will review some pertinent properties of the *E. coli* cell envelope, the known genetic lesions in fatty acid biosynthesis, and useful chemical inhibitors of fatty acid synthesis. Attention will then be focused on the phase properties of lipids, model systems from which these properties were derived, and the study of these properties in *E. coli* by using different physical techniques. A detailed discussion will deal with correlations between lipid physical properties and membrane-associated physiological processes. Lastly, we will address the problem of how *E. coli* regulates its lipid physical properties.

This review is intended to complement our previous review on membrane lipid metabolism (19) and thus will largely ignore this field. The metabolic alteration of fatty acids in other organisms, in some cases possibly for the purpose of regulating lipid physical properties, has recently been reviewed by Fulco (33).

### Pertinent Properties of the Cell Envelope

The cell envelope of *E. coli* consists of inner (cytoplasmic) and outer membranes (78). The inner membrane contains cytochromes, the enzymes of the electron transport system, the enzymes of phospholipid biosynthesis, and various proteins involved in active transport. In contrast, the outer membrane is almost devoid of known enzyme activities and contains the lipopolysaccharide component of the envelope. The envelope of *E. coli* contains all the cellular phospholipid, which is distributed between the inner and outer membranes. In addition to phospholipid, the outer membrane has two other lipid components, the lipid A moiety of the lipopolysaccharide (19, 78) and the murein lipoprotein (8, 40). The role of these structures in the function of the outer membrane is unknown (8, 78), although lipid A has recently been shown to be required for cell growth (78).

Since most of the studies discussed have examined inner membrane function or structure, we will define the work membrane as equivalent

to the inner membrane. In some of the physical studies to be discussed, the purity of the inner membrane preparation was not documented. Therefore, contamination of the inner membrane preparations with outer membrane may be a cause of some of the contradictory results obtained.

### *E. coli* Fatty Acid Auxotrophs

Since most of the data to be discussed are based on experiments with fatty acid auxotrophs of *E. coli*, a discussion of some properties of these strains is important.

The lesions causing unsaturated fatty acid (UFA) auxotrophy map in either of two genes, *fabA* or *fabB* (15, 16, 18, 25, 89, 92, 102, 103). *fabA* mutants are defective in  $\beta$ -hydroxydecanoyl thioester dehydrase (16, 102), the enzyme that introduces the double bond of the unsaturates (7), whereas *fabB* mutants have a defect in the condensing enzyme of fatty acid synthesis (89). Temperature-sensitive and absolute lesions have been reported in both genes. A third locus causing UFA auxotrophy is known, but the single existing mutant is quite "leaky" and has not yet been shown to be due to a defect in UFA synthesis per se (9). Some genetic and biochemical properties of UFA auxotrophs have been recently reviewed (103). Since both *fabA* and *fabB* auxotrophs are suitable (and have been used) for experimental manipulation of cellular fatty acid contents, we will refer to both simply as UFA auxotrophs.

UFA auxotrophs can be supplemented with a surprisingly wide variety of fatty acids. In general, most long-chain fatty acids having a hydrocarbon chain with a steric disorder in the central portion of the chain will suffice. Monounsaturated fatty acids with *cis* double bonds ranging from the 5 to the 13 position support growth, as will polyunsaturated fatty acids (21, 26, 28, 29, 69, 81, 100, 103). Various cyclopropane fatty acids (81, 100), branched-chain fatty acids (99), brominated derivatives of saturated fatty acids (27, 32), and *trans* unsaturated fatty acids (26, 28, 29, 81, 99-101, 103) will also support growth. The auxotrophs will grow at rates near normal on very unnatural fatty acids, although for some fatty acid supplements the growth temperature range is restricted (81).

Some unsaturated and branched-chain fatty acids will not support growth of UFA auxotrophs. This, however, is not necessarily absolute since variants of the original *fabB* auxotrophs had to be selected to allow supplementation with certain *trans* unsaturated (92, 122) and branched-chain fatty acids (99). The biochemical and genetic basis of these variant phenotypes is unknown. Conceivably, these mu-

tants might contain lesions at the level of either fatty acid transport (58) or activation (80). (Activation of the fatty acid by the acyl-coenzyme A (CoA) synthetase component of the  $\beta$ -oxidation system is a necessary step in fatty acid transport [58]). The mutants could also be altered in esterification of fatty acid into phospholipid.

In general, UFA auxotrophs are grown in defined medium supplemented with the potassium salt of an appropriate fatty acid (103). Usually a nonionic detergent is added to the medium to solubilize the fatty acid. Removal of UFA from the medium or change of UFA supplements is usually carried out by membrane filtration or centrifugation followed by washing with and resuspension in detergent-supplemented medium. It has been shown that no residual pool of fatty acid remains in the cell (76); hence starvation or fatty acid shift proceeds directly from the time of resuspension.

Auxotrophs of *E. coli* that require both saturated and unsaturated fatty acids have been isolated only recently (41, 42) and have not been extensively exploited. Penicillin selection was not successful in isolating such mutants, probably because a small amount of fatty acid synthesis is needed to provide the  $\beta$ -hydroxymyristate moieties of lipid A (41). Therefore, these strains were isolated as temperature-sensitive mutants by a [ $^3$ H]acetate suicide procedure (41). These mutants are able to grow at 37 C only if provided with either a *trans* UFA or a mixture of a saturated fatty acid and an unsaturated fatty acid (41). The biochemical lesion in one of these strains is in malonyl transacylase (42, 93).

### Specific Inhibitors of Fatty Acid Synthesis

Two inhibitors, 3-decynoyl-*N*-acetylcysteine (3-DNAC) (51, 52) and cerulenin (35), selectively inhibit fatty acid synthesis in *E. coli*.

3-DNAC is a specific inhibitor of UFA synthesis. This compound irreversibly inactivates the enzyme (that coded by the *fabA* gene [16]) that introduces the double bond of the unsaturated acids (7) and thus renders a treated culture phenotypically *fabA*<sup>-</sup> (51). Growth inhibition by low concentrations of 3-DNAC is reversed by addition of an appropriate UFA (51).

Cerulenin is an antibiotic that inhibits both unsaturated and saturated fatty acid synthesis in *E. coli* (35). Cerulenin specifically inactivates the condensing enzyme,  $\beta$ -ketoacyl acyl carrier protein synthetase, of fatty acid biosynthesis (20). Addition of this antibiotic to a culture of *E. coli* results in growth inhibition. Supplementation with both a saturated and an unsaturated fatty acid is required to reverse the inhibition (35). Therefore, addition of cerulenin

to *E. coli* results in a phenotype similar to that of mutants blocked early in the fatty acid biosynthetic pathway.

### LIPID BILAYER PHASE PROPERTIES

The temperature-dependent change of state of the acyl moieties of membrane phospholipids from a solid, hexagonal close-packing to a fluid, more random array has been termed a phase transition (24, 60, 96, 110). This process has also been called the order-disorder transition. At temperatures below the onset of the phase transition, the fatty acyl chains of the phospholipids are in a tightly packed, hexagonal array (Fig. 1). When the transition is complete, all chains are in a fluid state (Fig. 1). The temperature at the midpoint of the transition (the transition temperature,  $T_c$ ) and the temperature range between initiation and completion of the phase transition ( $\Delta T$ ) are both dependent on fatty acyl content and polar head group composition of the phospholipids.

#### Empirical Rules Deduced from Model Systems

A variety of physical studies in model systems for membrane bilayers have provided a set of empirical rules that can be used to relate fatty acid composition to order-disorder transition properties of lipids. Data illustrating some of the rules are given in Table 1. The more important rules are as follows.

(i) The  $T_c$  of lipids acylated with saturated fatty acids is higher than when the lipids con-

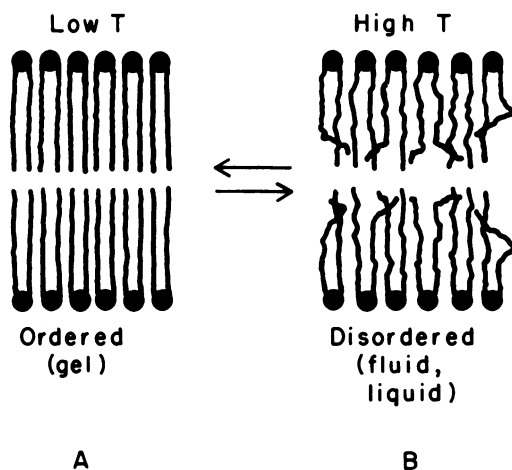


FIG. 1. Arrangement of fatty acyl chains in a liquid bilayer at temperatures below (low  $T$ ) or above (high  $T$ ) the extremes of the lipid phase transition. The phospholipid polar head group is denoted by the solid spheres. The acyl chains are denoted by the wavy lines.

TABLE 1. *Properties of phospholipid molecules*

Fatty acid	Chain length	Double bond position <sup>a</sup>	<i>T<sub>i</sub></i> of PC <sup>b</sup> derivative	Reference
<b>Saturated</b>				
Lauric	12	—	—2	22, 60, 83
Myristic	14	—	24	22, 60, 83
Palmitic	16	—	42	22, 60, 83
Stearic	18	—	58	22, 60, 83
<b>Monounsaturated</b>				
Oleic	18	9c	—22, 3 <sup>c</sup>	83, 84
<i>cis</i> -Vaccenic	18	11c	— <sup>d</sup>	
Palmitoleic	16	9c	— <sup>d</sup>	
Elaidic	18	9t	12 <sup>e</sup> , 26 <sup>e</sup>	83, 123
Palmitelaidic	16	9t		
<b>Polyunsaturated</b>				
Linoleic	18	9c, 12c	— <sup>f</sup>	
Linolenic	18	9c, 12c, 15c	— <sup>f</sup>	

<sup>a</sup> Symbols: *c* and *t* denote *cis* and *trans*, respectively.

<sup>b</sup> Unless otherwise noted both fatty acids in the phosphatidyl choline (PC) molecule are the fatty acid indicated. The transitions are those of bilayers in excessive water as measured by DSC.

<sup>c</sup> Transition of the derivative with stearic acid in position 1 (83).

<sup>d</sup> Results using other physical techniques (115) indicate the transition of these phospholipids would be similar to that of the dioleoyl lipid.

<sup>e</sup> Transition measured by TEMPO partition (123).

<sup>f</sup> We have not found direct DSC measurements of these transitions in the literature. However, from equivalent lipid packing experiments (115), transitions of less than -50 C would be expected.

tain *cis* monounsaturated fatty acids (compare distearoyl phosphatidyl choline with dioleoyl phosphatidyl choline). The presence of di- or polyunsaturated fatty acyl chains decreases the transition still further (60).

(ii) The *T<sub>i</sub>* of lipids substituted with homologous fatty acids is directly dependent on chain length (compare the saturated phosphatidyl cholines given in Table 1) (60).

(iii) Lipids containing *trans* UFA have a transition higher than that of the homologous *cis* unsaturated derivatives although below that of the homologous saturated derivative (60).

(iv) Lipids with fatty acids containing branched, cyclopropane, or other bulky side groups behave in a manner very similar to the same lipids containing the homologous *cis* UFAs (115).

(v) Heterogeneity of either chain length or saturation will broaden the range of the transition and may result in a phase separation (see below) (22, 60, 84, 111).

A useful visualization of these rules is provided by a schematic representation of the packing of lipids containing various fatty acyl moieties at a temperature below that of the phase transition (Fig. 2). The steric hindrance of the double bond results in a bilayer that has fluid character even at low temperatures.

The transition temperature is also dependent on polar group composition. For instance, the transition of dipalmitoyl phosphatidylethanol-

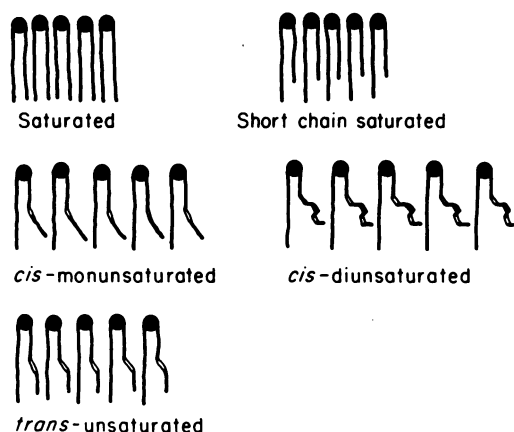


FIG. 2. Schematic representation of the fatty acyl chains below the beginning of the lipid phase transition. Depicted are phospholipid acylated with a 16 carbon fatty acid in position 1 of the *sn*-glycerol-3-phosphate backbone and fatty acids of the various types shown in position 2.

amine is 20 degrees higher than the transition of the homologous choline-derived lipid (60). The effect of polar head group composition is complex due to the charged nature of some groups and the ionic interactions of other groups (74, 111, 117). Therefore, no set of rules relating polar group composition to transition temperature is yet available.

The data given in Table 2 illustrate the rele-

vance of model system studies to an understanding of the properties of the *E. coli* membrane lipids. For instance, when an *E. coli* UFA auxotroph was grown on oleic acid, the membrane lipid transition was lower than when the auxotroph was supplemented with elaidate. Furthermore, supplementation with polyunsaturated fatty acids resulted in a membrane transition below that of the membranes from oleate-grown cells (on a per-UFA molecule basis). It should also be noted that the ranges of the transitions are increased if the physical properties of the incorporated unsaturate differ greatly from those of palmitate (the major fatty acid synthesized by UFA auxotrophs). For instance, the membranes from elaidate-grown cells have the most abrupt transition, whereas linolenate membranes have an extremely broad transition.

The amount of incorporation of a given fatty acid into phospholipids must be measured directly since various fatty acids are incorporated to different extents. For instance, Esfahani et al. (29) found that the lipid transition of cells grown on linolenic acid was higher than the transition of cells grown on oleic acid. This was surprising since the greater unsaturation of linolenic acid would be expected to produce a lower transition. However, examination of the fatty acyl content of the membrane phospholipids showed that linolenic acid was incorporated much less efficiently than was oleic acid. The difference in UFA content was therefore compensated by an increased content of satu-

rated fatty acids, and thus the transition of the linolenate-grown cells was much higher than that of the oleate-grown cells.

### Physical Properties of the *E. coli* Membrane Lipids

**Phase transitions.** The order-disorder transition can be detected in model systems by a variety of techniques. The methods that have been applied to *E. coli* membranes are X-ray diffraction, differential scanning calorimetry (DSC), spectroscopic monitoring of fluorescent probes, and a variety of electron spin resonance (ESR) techniques. We will discuss the results (given in Table 1) obtained with each of these methods. Our discussion assumes that the lipid in the inner membrane is structured into a lipid bilayer. Some direct evidence supports this assumption (120), and it is consistent with a large amount of other data (107).

(i) **X-ray diffraction.** This technique is the most direct method for examining the phase transition since it provides a quantitative evaluation (by measurement of distinct reflections) of the fraction of the hydrocarbon chains in the ordered and disordered conformations.

Two analyses of *E. coli* membranes using X-ray diffraction techniques have been published. Esfahani et al. (29) first demonstrated that the lipids of the *E. coli* membrane go through a lipid phase transition similar to that observed in *A. laidlawii* membranes. The transitions were found to be reliably dependent on fatty acid content ( $T_i$  for elaidate and oleate mem-

TABLE 2. Some lipid phase transitions observed in membranes of *E. coli* UFA auxotrophs<sup>a</sup>

Reference	Technique	UFA in membrane phospholipids							
		Elaidate <sup>b</sup>		Oleate		Linoleate		Linolenate	
		$T_i$	$\Delta t$	$T_i$	$\Delta t$	$T_i$	$\Delta t$	$T_i$	$\Delta z$
29	X ray	35	10	24	10	—	—	41	10
94	X ray	35	11	18	19	24	32	36	30
Mavis and Pastryk <sup>c</sup>	DSC	38	10	34	10	34	10	—	—
39	DSC	38	20	None detected		—	—	—	—
82	Fluorescence	38	4	15	13	—	—	—	—
91	Stearic acid spin label (C12)	37	3	17	4	—	—	—	—
91	TEMPO	37	7	—	—	—	—	—	—
66	TEMPO	38, 32	—	31, 14	—	28, 7	—	—	—
65	Hydrocarbon spin label	30	—	16	—	28, 9	—	—	—

<sup>a</sup> In all the studies listed, the fatty acid compositions of the membrane phospholipids agree well (variation of <10% of the total fatty acid) for membranes of cells grown on a given UFA. Transitions are given only for those membranes for which the fatty acid composition is known.

<sup>b</sup>  $T_i$  and  $\Delta t$  stand for the transition temperature and range, respectively. The temperature are degrees centigrade.

<sup>c</sup> R. D. Mavis and S. A. Pastryk, Fed. Proc. 34:668, 1975, and R. D. Mavis, personal communication. The values given are for cell envelopes. Envelopes whose phospholipids contained only 26% oleic acid had a  $T_i$  of 40°C ( $\Delta t = 12^\circ\text{C}$ ).

branes were 35 and 24 C, respectively). These results were recently extended by Shechter et al. (96). Use of a novel diffractometer has allowed these later workers to make much more sensitive measurements (23) than were previously possible. Thus Shechter and co-workers (94) have measured the early part of the phase transition which was inaccessible to Esfahani and co-workers (29). These new data describe an increased range for the transition. Despite this discrepancy, there is good agreement between the two studies. The data for elaidate-substituted membranes agree well, as do the upper limits of the transition, with the data for other fatty acid supplements. Shechter et al. (96) also observed a hysteresis with membranes from linolenate-supplemented cells that was probably due to a lipid phase separation within the membrane (see below). In both of these studies the membranes and the corresponding isolated lipids gave very similar transitions, indicating that lipid conformation was indeed being measured.

(ii) DSC. Although less direct than X-ray diffraction, has provided much valuable evidence in other systems. However, rather little work on the *E. coli* envelope has been done by using this technique.

DSC measures the differential heating uptake between the experimental sample (i.e., membranes in buffer) and a reference sample (buffer) as both samples are simultaneously heated (109). This method gives unequivocal results in model systems in which the molecules absorbing heat are known. However, in biological membranes it is often difficult to ascertain which molecules are absorbing heat. Therefore, measured lipid transitions may also include heat uptake by other membrane molecules (e.g., protein). The *E. coli* membrane is said to be particularly difficult to study by DSC (109). However, some data are available (39, 108; R. D. Mavis, personal communication) that agree fairly well with the appropriate X-ray diffraction results. An exception to this statement is the phase transition observed in wild-type *E. coli*. Steim (108) reports the DSC-measured transition in such membranes to extend from about 0 to 37 C, whereas X-ray diffraction results of Shechter et al. (95) give a transition for a similar preparation extending from about 20 to 30 C. The samples examined by X-ray diffraction, however, were partially dehydrated, which tends to raise the phase transition. It should also be noted that base-line problems encountered with most commercial DSC instruments make the detection of transitions in heterogeneous mixtures of lipids difficult. Indeed, Haest et al. (39) were unable to

detect the lipid phase transition of membrane preparations from an oleate-grown UFA auxotroph. This base-line problem is most apparent at the lower end of the phase transition. Such instrumental problems may explain the results of Mavis and Pastryk (Table 1). They found DSC transition for oleate and linoleate membranes that were considerably higher than those observed by the other techniques.

(iii) Fluorescence probes. The most extensive data on the phase transition of *E. coli* membranes and lipids is that of Overath, Träuble, and coworkers (82, 91, 112). These workers have studied the transitions in membranes with fluorescence probes and in lipids by a large variety of techniques. The fluorescence technique measures the amount of disordered lipid mainly as a result of a preferential partitioning of a fluorescent dye into the hydrocarbon phase of the bilayer when it is in a fluid state. Partition of the dye into hydrocarbons causes a large increase in fluorescence intensity. Low concentrations of the dye do not influence the phase transition (82, 112). The fluorescence measurements of Overath and Träuble agree rather closely with the X-ray results of Shechter et al. (96) (Table 1). A direct comparison of the two techniques gives very close agreement (P. Overath, personal communication; 78a). Calibrations reported by Träuble and Overath (112) indicate that the fluorescence technique monitors the state of about 80% of the membrane lipids. Therefore, the fluorescence spectroscopic technique (like X-ray diffraction and DSC) measures the average phase transition of the membrane. The transitions observed in the lipids isolated from these membranes agree very well with the transitions observed in the membrane preparations from which the lipids were derived (82, 91, 112).

The results given by the X-ray, DSC, and fluorescence spectroscopy techniques, therefore, indicate that the lipids of the *E. coli* membrane go through a classical order-disorder transition similar to that idealized in Figure 1. However, evidence for phase separation in addition to the order-disorder transition has also been reported.

(iv) Spin-labeling techniques. Electron paramagnetic resonance of spin labels either partitioned or incorporated into the membrane usually gives results that differ from those obtained by X-ray diffraction. These results have been interpreted largely by postulating lateral phase separations and will be discussed below.

**Phase separation.** (i) Physical techniques. A phase separation in a lipid bilayer is defined as a lateral movement of lipids to form (under

equilibrium conditions) regions enriched in similar lipid molecules. This movement is followed by a discrete solid-to-liquid phase transition for each region. Strong evidence for phase separations has been obtained in model systems (22, 47, 83, 84, 117). Although many workers have interpreted various physical findings as proof of phase separations in *E. coli* membranes, correlation of the data with the behavior of model systems casts doubt on some of these conclusions.

Shechter et al. (96) have recently presented compelling evidence for the existence of lipid phase separations in *E. coli* membranes. Using X-ray diffraction, these workers found that both the membranes and the isolated lipids from UFA auxotrophs supplemented with linolenic acid show hysteresis in their temperature-dependent phase transitions. In these membranes two phase transitions were detected by X-ray diffraction, one at 10 to 15 C and the other at 25 to 45 C. Model system studies (22, 47, 83, 84, 117) and the fatty acid composition of these membranes permit the lower transition (10 to 15 C) to be attributed primarily to lipids containing one acyl chain each of palmitate and linolenate, whereas the higher transition would be correlated mainly with dipalmitoyl phospholipids. This interpretation adopts the model systems' assumption that the two transitions seen in the mixture would occur at temperatures between those observed for the two pure components (dipalmitoyl- and 1-palmitoyl,2-linoleoyl phospholipids).

Using ESR and the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), McConnell and co-workers (57, 66) have detected two discontinuities in plots of the TEMPO parameter,  $f$ , versus temperature for *E. coli* membranes isolated from an auxotroph grown on either oleate, elaidate, or linoleate. The parameter  $f$  is said by Shimshik and McConnell to be "approximately equal to the fraction of molecules of TEMPO dissolved in the fluid hydrophobic region of the membrane" (97). Due to the similarity of these plots to those observed in binary model systems (36, 97), these workers have interpreted the two breaks observed in *E. coli* membranes as evidence for phase separation. However, the X-ray technique used by Shechter et al. (96) did not disclose phase separations in the membranes or the lipids from cells grown on oleic, elaidic, or linoleic acids. Therefore, these X-ray data contradict the work of Fox, McConnell, and co-workers (57, 65, 66), who report phase separations to occur in membranes from cells grown on these acids. We believe that this contradiction may be due to difficulties in the interpretation of TEMPO partitioning in complex systems.

In a series of studies on model systems, McConnell's laboratory (36, 97, 123) has interpreted the higher temperature discontinuity of the TEMPO data as indicating the onset of phase separation and the lower discontinuity as the completion of phase separation. This interpretation clearly depends on what a discontinuity in a plot of TEMPO partition versus temperature denotes in molecular terms. There is no doubt that TEMPO can detect accurately the classical lipid phase transition in single-component model systems (49, 97). However, the interpretation of TEMPO data in model systems of two components (and hence in biological membranes) is controversial. McConnell and co-workers have shown that it is possible to interpret such data in terms of lipid phase separations (36, 97). However, Lee et al. (61) have shown that TEMPO detects a discontinuity in single-component model systems that is not attributable to the order-disorder transition or to phase separation (since there is only one component). Similar data have subsequently been published by Wu and McConnell (123). Lee and co-workers interpret this discontinuity as evidence of lipid cluster formation. A lipid cluster is envisioned as a short-lived, more densely packed arrangement of lipid molecules (which are not necessarily of similar composition) within an environment of freely dispersed molecules. These authors suggest that TEMPO data from mixed model systems might be more readily interpreted as a steady state of metastable lipid clusters rather than as an equilibrium-obtained phase separation. These authors cite a large number of observations in both model and biological systems to support their claim that cluster formation may be a major feature of the disordered state of the lipids.

A comparison of phase separations in model systems as observed by TEMPO and by DSC tends to support the contentions of Lee et al. (61). For instance, Shimshik and McConnell (97), using the ESR-TEMPO technique, have reported data interpreted as phase separations in equimolar mixtures of dimyristoyl lecithin and dipalmitoyl lecithin. However, two other groups using DSC found no evidence for phase separations in the same mixture of phospholipids (22, 83, 84). DSC did detect phase separations only for mixtures of lecithins having acyl chain lengths differing by greater than four carbon atoms (22, 83, 84). It should also be noted that the ESR measurements of *E. coli* UFA auxotroph membranes reported by Linden and her co-workers (66) are clearest for those *E. coli* membranes containing elaidate. Phosphatidylcholine esterified with this fatty acid gave aberrant ESR results with TEMPO in model systems (123). Similar results are seen with the

dioleoyl lipid (61). These aberrancies are those interpreted as evidence for lipid clustering by Lee and co-workers (61).

A series of ESR experiments performed with spin-labeled stearic acids or hydrocarbon derivatives do not clarify the ESR results with TEMPO. Sackmann et al. (91) used a series of spin-labeled stearic acid derivatives designed to probe various regions of the acyl chain. Those probes having the spin label close to the methyl end of the chains gave transition temperatures similar to those reported by Overath and Träuble (discussed above). A probe located close to the carboxyl group gave slightly lower transitions.

Linden et al. (65) have reported results with spin-labeled hydrocarbons that were also designed to probe the nonpolar region of the bilayer. Their results differ from TEMPO results with the same strain of *E. coli*. Cell membranes supplemented with oleic acid or with elaidic acid gave only a single transition which corresponded to the lower transition were detected by the hydrocarbon probes. The transition of the oleic acid membranes agreed with that obtained by the averaging methods (e.g., X-ray diffraction); however, the elaidate  $T$ , did not.

In conclusion, the lipids of the inner membrane of *E. coli* have been shown to undergo a classical order-disorder phase transition as the temperature is increased. At this time, it seems most likely to us that lipid phase separations occur on a large scale in the *E. coli* membrane only when the lipids are extremely heterogeneous in their acyl composition. Since the lipids found normally in *E. coli* are rather homogeneous in their properties (19), it then follows that large-scale phase separation does not occur in the normal cell. To reconcile this view with the results of Linden and her co-workers (66), we suggest that the TEMPO label used by these investigators may not be measuring large-scale lipid phase separations in *E. coli* membranes. This spin-label technique may be a probe for heterogeneity since it could preferentially partition into special membrane environments. It is also possible that the spin label measures the properties of those membrane lipids whose conformation has been disturbed by interaction with the label (for an example see reference 11). The TEMPO results could, therefore, be due to either phase separation or some other perturbation on a small scale that encompasses some minor region of the membrane. In this regard, it should be noted that the temperature at the lower TEMPO inflection observed in *E. coli* membranes often did not correlate well with the analogous inflection temperature observed in the isolated membrane lipids (66). In fact, with lipids isolated from linoleate-supple-

mented cells, essentially no lower inflection was seen (66). The amount of TEMPO partitioning in the hydrophilic region at the lower inflection was two to three times greater in membranes than in the isolated lipids. Together these anomalies suggest that TEMPO may be sensing membrane changes other than those due to the lipid component (i.e., partition into hydrophobic protein). It seems reasonable that the upper TEMPO inflection could be due to lipid clustering as suggested by Lee and co-workers (61).

(ii) **Freeze-fracture electron microscopy.** Another technique yielding data consistent with the concept of phase separation is freeze-fracture electron microscopy. Three laboratories have presented data indicating that the lipid composition and temperature (before freezing) of the membranes from UFA auxotrophs can change the distribution of particles (presumably protein) within the membrane (39, 57, 94). In general, membranes frozen from temperatures above the phase transition have a somewhat less random pattern of particles, whereas cells frozen from temperatures below the onset of the phase transition show an aggregation of the particles with large smooth areas between the aggregates. The most detailed study, that of Kleeman and McConnell (57) on elaidate-supplemented membranes, indicated that aggregation occurred within an narrow temperature range that corresponded closely to the inflection of the lipid phase transition. However, when membranes from cells grown on other UFA are examined, this correlation breaks down. For instance, Shechter and co-workers (96) found aggregated particles and large smooth areas in linolenate membranes frozen from 25 C, a temperature well within the overall lipid phase transition of these membranes. Kleeman and McConnell (57) observed aggregates in oleate membranes frozen from 22 or 25 C. These temperatures are also within the phase transition of the membranes. Another puzzling observation is the difference (57) in the patterns observed between wild-type cells (a random pattern) and a UFA auxotroph grown on oleic acid (partially aggregated). The lipid composition of the auxotroph is similar to (in fact, slightly more homogeneous than) that of a wild-type strain (65, 98). Thus the particle distribution of wild-type and oleate-grown cells should be the same at a given temperature if lipid composition is a chief determinant of the freeze-fracture patterns.

For these reasons it is difficult to interpret these freeze-fracture patterns solely in terms of lipid phase separations. The freeze-fracture technique may select different regions of membranes in cells of differing lipid composition or

there may be other technical problems. In fact, the patterns of membranes from wild-type cells of *E. coli* differ greatly from laboratory to laboratory (for review see reference 57).

(iii) **Lateral movement of lipids.** Two measurements of the rate of lateral motion in the *E. coli* membrane have been reported. Unfortunately, the results of the two experiments are quite dissimilar.

From a spin-label study similar to that done in lipid vesicles, Sackmann and co-workers (91) found the lateral mobility of lipid in the *E. coli* membrane (above the phase transition) to be  $3.25 \times 10^{-8}$  cm/s. This result is similar to that found in synthetic lipid vesicles, and thus the average distance of travel per s would be 2.7  $\mu$ m. Since the average length of an *E. coli* cell can be taken as 1 to 2  $\mu$ m, this rate of travel is sufficient to allow a lipid molecule to travel from one end of the bacterium to the other in less than 1 s.

However, Dupont et al. (23) have reported an experiment examining the disorder-to-order transition of the acyl chains of the lipids in *E. coli* membrane vesicles. They find the rate of the appearance of order to be several orders of magnitude slower in the *E. coli* membrane than in a liposome. If the average lipid molecule moved at a rate calculated from these data, it would take a lipid molecule on the order of hours to move from one end of an *E. coli* cell to the other.

The literature, therefore, gives two values for lateral diffusion of lipids in *E. coli* that differ by several orders of magnitude. Both estimates involve assumptions. The estimate of Sackmann et al. (91) assumes that the spin-label resides in the average domain of the *E. coli* membrane lipid. The X-ray value (23) assumes that segregation of lipids according to the type of acyl chains must occur before the disorder-order transition can occur in the *E. coli* membrane. Clearly, one of the two assumptions must not be valid. In the absence of additional data we cannot say which of the two this is.

#### CORRELATIONS BETWEEN MEMBRANE-ASSOCIATED PHYSIOLOGY AND LIPID PHYSICAL PROPERTIES

##### General Physiology

Overath and co-workers (81) have shown that a number of metabolic parameters can be affected by manipulating the UFA content of *E. coli*. Growth, respiration, and efflux of thio-methylgalactoside were compared with the phase transitions observed in phosphatidylethanolamine isolated from cell membranes. The

inflection points observed in Arrhenius plots for each of these functions agree well for cells grown on a given fatty acid. The transition temperatures observed for cells grown on different fatty acids varied as expected from model studies. For instance, elaidate-grown cells showed characteristic temperatures of about 38 C, whereas the characteristic temperatures of oleate-grown cells were 10 to 15 C. Thus, several physiological properties seem dependent on the phase properties of the membrane lipids.

##### Lactose Transport System

Manipulation of the UFA content of *E. coli* auxotrophs has been used in a number of attempts to probe the genesis and functional requirements of the lactose transport system.

**Relevant properties of the lactose transport system.** The lactose transport system consists of a protein (the M protein) coded by the *y* gene of the lactose operon (which also codes for  $\beta$ -galactosidase and thiogalactoside transacetylase) and an ill-defined energy coupling system (53, 59). The M protein is known to be located in the inner membrane, and components of the energy coupling system are also thought to be membrane bound (53). The absolute energy requirement for transport system function has only recently been demonstrated (59).

It should be noted that the requirement of lactose transport for an energy supply has largely been ignored in the literature to be reviewed. The laboratories engaged in studying the relationship between membrane synthesis and lactose transport prefer to interpret their data in terms of effects on the M protein component of the system. This may well be true; however, it remains to be established that effects on energy production or coupling are not involved. Attempts to control for this parameter have involved assay of the other lactose operon products or the assay of other "unrelated" transport systems. Neither method suffices. The first fails because the other operon products are enzymes that do not require energy for their function. The second, the use of genetically unrelated transport systems, suffers from the criticism that only the recognition component of the transport system is known to be genetically distinct from lactose transport. Transport of various sugars may utilize different recognition proteins whose functions are coupled to the same energy supply. Indeed, the phosphotransferase system of sugar transport functions in precisely this manner (note that lactose is not transported by the phosphotransferase system; for review see reference 63).

**Transport system induction and lipid synthesis.** Studies of the relationship between phospholipid synthesis and induction of a functional lactose mechanism have used two approaches. In the first series of experiments, Fox and co-workers (31, 48) concluded that assembly of a functional transport system required concurrent normal lipid synthesis. This conclusion spurred much of the work to be discussed below. The original experiments involved a disruption of normal lipid synthesis by starvation of the appropriate auxotrophs either for the unsaturated fatty acid or for the glycerol required for normal phospholipid. However, extensive experiments in several laboratories (76, 79, 87, 118) have failed to repeat these observations.

For instance, Nunn and Cronan (76) found that induction of lactose transport was normal in cells starved for UFA by several different methods. No inhibition of lactose transport induction was observed under conditions allowing only about 4% of the normal rate of UFA synthesis. The original results of Fox (31) are best explained by assuming that transport induction was assayed after extensive damage to the cells (76). Furthermore, Weisberg et al. (118) have also reported experiments with two classes of glycerol auxotrophs which indicate that a profound inhibition of phospholipid synthesis has no preferential effect on the induction of lactose transport. Therefore, this approach has not yielded data useful to understanding transport system biogenesis.

**Transport and lipid phase changes.** Another approach to studying the biogenesis of the lactose transport system was adopted by Wilson and Fox (121). This approach was based on the finding (92, 122) that the rate-temperature profile of lactose transport could be altered by supplementation of UFA auxotrophs with various UFAs. The Arrhenius plots for transport were found to be biphasic (Fig. 3). The two line segments could be extrapolated to an intersection point that was dependent on the UFA used to support growth. The effects of several fatty acid supplements were tested in temperature studies of both  $\beta$ -glucoside and lactose transport systems. The inflection temperatures observed varied widely from 7°C for linoleate to 30°C for elaidate. The authors, therefore, suggested that the Arrhenius plot inflections are an indication of a transition in the lipid environment of transport sites in the membrane.

Wilson and Fox (121) then attempted to exploit the fatty acid-dependent temperature profile of lactose transport activity to study the assembly of the transport system. Their approach was to shift fatty acid supplements be-

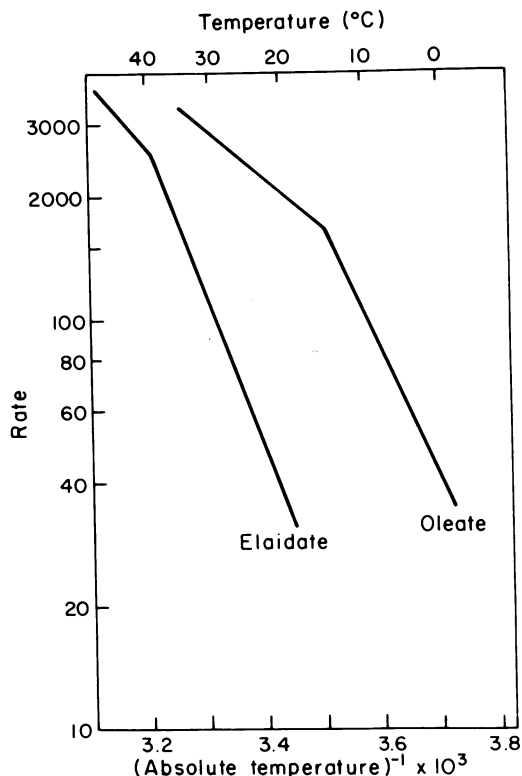


FIG. 3. Hypothetical Arrhenius plot similar to those reported for transport of various sugars and amino acids.

fore or after induction and determine whether the lactose transport system had the temperature profile of the first or second fatty acid. These workers found the lactose transport system to have the temperature profile of the fatty acid present during (rather than before or after) induction and thus concluded that newly synthesized proteins associate preferentially with newly synthesized lipids.

Overath and co-workers (79) studied the temperature characteristics of the lactose transport system in an *E. coli* UFA auxotroph grown in medium supplemented with either oleate or palmitelaidate. Their results differed greatly from those of Wilson and Fox (121). Overath et al. (79) grew the auxotroph in medium supplemented with one fatty acid and then shifted the organism to medium supplemented with the other fatty acid for various periods of growth before lactose operon induction. In addition, cells growing with oleate or palmitelaidate were induced and subsequently shifted to media lacking inducer but containing palmitelaidate or oleate, respectively. Temperature studies of lactose transport activity in all instances indicated that the transition points

observed in the Arrhenius plots were dependent upon the average membrane fatty acid compositions rather than the fatty acid composition of the phospholipids synthesized during transport induction.

This conflict has recently been resolved. Tsukagoski and Fox (114) have reported data in general agreement with those of Overath et al. (79) and report that the conclusions of Wilson and Fox (121) were the result of an inadequate characterization of the rate-temperature profiles.

However, Tsukagoski and Fox (114) also reported that when transport induction was measured at 25 C after shifting from growth with an oleic acid supplement at 37 C to growth with an elaidic acid supplement (or vice versa), the Arrhenius plots were found to be triphasic. Two discontinuities were observed, one at the temperature at which the single discontinuity was found for elaidate-grown cells and the other at the single temperature discontinuity seen in oleate-grown cells. A short incubation at 37 C of the cells induced at 25 C gave the same result as cells induced at 37 C (i.e., only a single discontinuity between the two extremes). These data were interpreted as indicating that low temperatures allow the observation of the association of newly formed transport protein with newly synthesized lipid. Tsukagoski and Fox (114) also suggested that such a preferential association might also occur at 37 C (as proposed by Wilson and Fox [121]) but the rapid randomization (in the presence of the increased temperature) of newly synthesized lipids among previously existing lipids precludes its observation at the higher temperature.

We have considerable reservations concerning the validity of this interpretation. First, the cells were not growing and in some cases were losing viability during the induction period. Second, the data interpreted as showing three slopes (two discontinuities) on an Arrhenius plot can be readily accommodated by two slopes. The three-slope (versus two-slope) interpretation depends only on one or two points per graph, some of which were measuring very low transport rates with an attendant poor signal-to-noise ratio. Third, and most confusing, are some later experiments from the same laboratory using the same UFA auxotroph (64, 66). The original Arrhenius plots of Wilson and co-workers (121, 122) showed only a single discontinuity for transport induced in cells supplemented with either elaidate (30 C) or oleate (13 C). The results of Linden et al. (66) confirm an inflection point for elaidate-supplemented cells at 32 C and show a further discontinuity at 38 C. However, the interpretation of Tsuka-

goski and Fox depends on the simple picture of one characteristic transition (114). Most recently, Linden and Fox (64) have reported that cells supplemented with elaidate or oleate reveal, respectively, two or three discontinuities with rate-temperature profiles of transport. We feel that these reservations render the observations of Tsukagoski and Fox (114) essentially uninterpretable.

Tsukagoski and Fox (113) have also reported experiments from which they conclude that induction of transport in cells incubated at temperatures below the transition temperature of their membrane lipid is abortive. They found that the ratio of transport induction to  $\beta$ -galactosidase induction decreased dramatically as the temperature during induction was decreased. The induction temperature at which they first observed this decrease was dependent on the fatty acid composition of the cell (28 C, 13 C, and 20 C for elaidate-, oleate-, and bromostearate-grown cells, respectively). We feel that the interpretation of these results is complex. First, from the results of the companion paper by Tsukagoski and Fox (114) and the results of others (28, 81), elaidate-grown cells respond to a temperature decrease by halting growth, losing viability, and (under some conditions) by lysis. Since Tsukagoski and Fox (114) induced their cultures under such conditions, induction of a membrane transport system could be attributed to a general lack of membrane synthesis or to membrane or other cellular damage. A lack of membrane synthesis (or membrane damage) could be expected to affect induction of transport more than the induction of  $\beta$ -galactosidase. It is, therefore, possible that simply stopping growth is sufficient to cause an altered ratio of transport induction to  $\beta$ -galactosidase induction. Also disturbing is the finding that bromostearate- and oleate-grown cells showed similar behavior in these physiological experiments, whereas elaidate-grown cells appeared quite different. However, physical studies from the same laboratory (65) are interpreted as showing that the membranes from elaidate- or bromostearate-grown cells have rather similar lipid phase properties. Therefore, the interpretation of these data is unclear.

As discussed above, Overath and co-workers (82, 91) found that the inflection points observed for transport agreed well with the order-disorder transitions observed for the membrane lipids and in membranes. These workers find only a single inflection in Arrhenius plots of lactose transport. However, later experiments of Fox and co-workers (64-66) now report multiple inflections in Arrhenius plots of lactose transport. Overath and co-workers (82, 91) did

not find such behavior, but their studies are neither as detailed nor cover as wide a temperature range as the recent results of the Fox laboratory (64–66), and this may explain the discrepancy. The results of Fox and co-workers (64–66) are interpreted in terms of phase separations (see above). For the following reasons, we feel it possible that some of these inflections are not due to lipid phase changes. First, in oleate-supplemented cells, multiple inflections are often seen at temperatures above 20 C. The inner membranes from oleate-grown cells are quite fluid since most (>99%) of the lipids contain at least one oleate moiety (65, 98). Such lipids have transitions beginning below 20 C and do not seem likely to cause inflections above the transition. Second, the sharpness of some of the transitions reported for elaidate-grown cells seems too abrupt to be membrane lipid changes, especially if the sharpness of the transition is dependent on the size of the cooperative unit (number of lipid molecules) involved in the transition (46). Third, the number of transitions does not appear related to the complexity of the lipid acyl composition in any simple manner.

A direct comparison of the lactose transport results of the Overath and the Fox groups is possible for cells grown on both elaidic and oleic acid since the appropriate fatty acyl compositions have been reported from both laboratories. For elaidic acid, the single lactose transport inflection observed by Overath and co-workers (82, 91) agrees well with the higher inflection observed by Fox and co-workers (64, 66). However, for oleic acid, the single Overath inflection correlates with the lower inflection observed by Fox. This direct comparison of the fatty acid compositions, therefore, gives no clue to explain the differences in the inflection points for lactose transport reported by these two laboratories.

### Other Transport Systems

**Sugar transport systems.** Fox and co-workers have often studied the  $\beta$ -glucoside transport system of *E. coli* in parallel with their studies on the lactose transport system (64–66, 121, 122). The results obtained for these two systems are almost identical and have been interpreted in the same manner; they are thus sufficiently discussed above.

The transport of glucose which is mediated by the phosphotransferase system (63) is not affected by changes in the lipid phase transition (96).

**Amino acid transport systems.** The influence of lipid phase transitions on amino acid transport has been examined in several labora-

tories. Both Esfahani et al. (29) and Shechter et al. (96) have examined proline uptake by membrane vesicles. Vesicles prepared from UFA auxotrophs grown with various UFA supplements were examined. Both laboratories found single discontinuities in the Arrhenius plots for proline uptake by vesicles containing oleic, linolenic, or elaidic acid. The results obtained by both laboratories with oleic and linolenic membranes are similar, although the inflections occur at temperatures above those observed for lactose transport. A comparison of the data from the two laboratories that were obtained for elaidate grown cells is puzzling. Although the X-ray transitions and fatty acid compositions of elaidate vesicles are almost identical, Esfahani et al. (29) report the inflection point for proline transport as 26 C whereas Shechter et al. (96) report a 38 C inflection (similar to that obtained for lactose transport).

Sketchy data for glycine and arginine transport suggest inflection points for both transport systems similar to those of lactose transport (88). The effect of membrane fatty acid content on amino acid transport may deserve further exploration since progress is being made on the energetics of transport in these systems (6).

### Other Physiological Processes

Several laboratories have studied the relationship between various cellular processes and either UFA synthesis or the lipid phase transition. UFA starvation of *E. coli* results in the synthesis of lipids containing two saturated fatty acids (98). This altered synthesis would result in an increased phase transition and perhaps in phase separation. Therefore, UFA starvation might inhibit cellular functions by either (i) decreasing the availability of phospholipids containing UFA which are required cofactors for enzymatic or other activity or (ii) raising the lipid phase transition. Most of the papers to be discussed allow no choice between these two alternatives.

**Lipid requirement for alkaline phosphatase derepression.** Izui (50) reported that during UFA starvation no alkaline phosphatase activity was formed in response to phosphate limitation. These experiments are incomplete due to two omissions. First, Izui did not show that normal derepression of the *phoA* gene occurred in UFA-starved cultures. Second, cells having an ordered membrane lipid are known to release less of various noninducible periplasmic enzymes (88), and thus the effect may not be related to derepression. Experiments by D. F. Silbert and M. Schlessinger (personal communication) showed that alkaline phosphatase

tase is formed quite normally during UFA starvation of a constitutive strain.

**Chemotaxis.** Lofgren and Fox (67) have recently reported data indicating that *E. coli* requires fluid membrane lipids for chemotaxis but not for motility. A UFA auxotroph grown on elaidate exhibits normal chemotaxis at 32 or 35 C but does not show chemotaxis at 25 or 28 C although motility is similar at all four temperatures. In contrast, oleate-grown cells show normal chemotaxis at all four temperatures. The fact that elaidate-grown cells exhibit normal chemotaxis indicates that the methionine requirement for chemotaxis does not involve cyclopropane fatty acid formation.

**Initiation of DNA synthesis.** Fralick and Lark (30) have recently reported a series of convincing experiments indicating that UFA synthesis is required for the initiation of deoxyribonucleic acid (DNA) synthesis. These workers showed that in the presence of 3-DNAC, growing cultures of *E. coli* completed a cycle of DNA replication but were unable to initiate further rounds of replication. This result was reversed by addition of oleic acid and furthermore was shown to be unrelated to the cessation of ribonucleic acid and protein synthesis also caused by the inhibitor. Mutants specifically defective in initiating DNA replication were found to be unable to replicate DNA at a permissive temperature if replication had been terminated at a nonpermissive temperature in the presence of 3-DNAC. These mutants also became more temperature sensitive if the shift from a nonpermissive to a permissive temperature was performed in the presence of 3-DNAC. In both studies, only DNA initiation mutants were affected by 3-DNAC; mutants defective in DNA chain elongation were unaffected. All the effects could be reversed by addition of UFA. These results suggest a model in which replication is initiated at a particular lipid-associated site on the membrane. In the absence of UFA synthesis (or in the presence of a higher lipid transition—see above), functional initiation sites cannot be formed.

**Methylgalactoside permease induction.** Robbins and Rotman (87) have reported that concurrent synthesis of UFA must occur to permit normal induction of the methylgalactoside permease. These workers found no induction of transport if UFA synthesis was blocked (by addition of 3-DNAC) during the induction period. Addition of UFA reversed the effect of 3-DNAC addition. Transport was measured by accumulation of radioactive sugar, but controls for efflux were not given. The results could therefore be due to masking of transport by

efflux of the accumulated sugar. In this regard, the finding that inhibitor addition results in a rapid and substantial decrease in the basal level of the permease activity may be instructive, especially since the transport system was not rapidly degraded upon addition of the inhibitor. Thus, the interpretation of these interesting results does not seem clear and further experiments are needed to clarify the role of UFA synthesis in the formation of this complex transport system.

**Cell integrity.** Henning and co-workers (45) first showed that UFA auxotrophs lyse upon prolonged starvation for UFA. This lysis was most striking in rich media. This is probably due to the faster growth rate supported by rich media since growth is required for lysis (45). The starved cell also becomes leaky to several otherwise impermeable substrates just before lysis begins (76, 79, 100, 108). The requirement for growth suggested that lysis was due to the synthesis of abnormal lipids containing two saturated fatty acids (98) and thus changing the phase properties of the membrane. This was confirmed by our finding (discussed below) that lysis begins when the UFA content of the cell declines below the minimum value allowing growth (16).

Two laboratories have recently reported that an osmotic stabilizer added to the growth media allows supplementation of an auxotroph by 8 to 10% UFA as opposed to the 15 to 20% UFA found in standard media (2, 10). These results suggest that one of the major functions of a fluid membrane may be osmotic stabilization.

**Enzymatic activity.** Mavis and Vagelos (72) examined the rate-temperature profiles for three membrane-associated enzymes from UFA auxotrophs supplemented with various fatty acids. Membrane preparations from cultures grown with oleic or linolenic acid showed similar Arrhenius plots for *sn*-glycerol-3-phosphate acyltransferase, although the phase transitions of such membranes are quite different. These plots showed no abrupt breaks such as those observed for lactose transport. However, the enzyme activity in elaidate-substituted membranes did show a discontinuity. 1-Acylglycerol-3-phosphate acyltransferase showed a difference in slope between membranes from cells grown on *trans* or *cis* acids, but no breaks were observed. A third membrane-associated enzyme, *sn*-glycerol-3-phosphate dehydrogenase (catabolic), seemed unaffected by fatty acid manipulation. These results were interpreted as suggesting that the acyltransferases were dependent on the state of the lipid but the dehydrogenase was not. This interpretation was sup-

ported by the subsequent finding of Mavis et al. (71) that phospholipase C treatment of membranes decreased the activity of both acyltransferase activities but had no effect on the dehydrogenase activity.

The conclusions of Mavis and co-workers (71, 72) were strengthened by the subsequent studies of Esfahani and co-workers (27, 29). Arrhenius plots of the succinic-ubiquinone reductase activity of *E. coli* membranes from supplemented UFA auxotrophs exhibited single discontinuities (29). The inflection temperatures in these plots were dependent on the UFA supplied to the cells. These temperatures, however, were either at or below the beginning of the X-ray transition of the membrane (29).

These authors subsequently reported more direct and compelling evidence on the effects of phospholipid alterations on this enzyme activity (27). Extraction of the membranes with acetone removed about half of the envelope phospholipids and resulted in a 70% loss in enzymatic activity. Readdition of total cell lipids, consisting of phospholipid and coenzyme Q, restored activity to normal. Neither cell lipid component restored activity when added alone. After acetone extraction, both oleate- and elaidate-supplemented membranes exhibited continuous Arrhenius plots for the enzyme in question. The reactivation by total cell lipids restored the UFA-dependent discontinuities. Neither isolated cell lipids nor coenzyme Q alone was able to restore the discontinuities in the Arrhenius plots. The data, therefore, indicated that the inflections in Arrhenius plots of succinic-ubiquinone reductase were dependent on phospholipid acyl group composition. The temperature of the inflection was acyl group dependent; however, this relationship was not simple. For a given fatty acid composition, the inflection temperature found in the reactivated membranes was several degrees above that found in the native membrane and correlated in only a general way with the lipid transitions measured by X-ray diffraction. For example, the inflection point observed for this activity in native elaidate-supplemented membranes was 22 C, 27 C in reactivated preparations, both far below the beginning of the phase transition observed in these membranes. However, the inflection observed in oleate membranes, 11 C (18 C in reactivated preparations) was in the midst of the lipid phase transition of these membranes. The resolution of these discrepancies may lie in the heterogeneity of the lipid fatty acyl composition.

Experiments similar to those of Mavis and Vagelos (72) were reported by Beacham and

Silbert (3) on the uridine diphosphate-galactose-lipopolysaccharide galactosyl-transferase. This enzyme is known from the studies of Rothfield and Romeo (90) to require phospholipids containing UFA for activity and thus might be expected to provide more easily interpretable results. Beacham and Silbert (3) found that Arrhenius plots of this enzyme activity in membranes grown on *trans* UFAs were linear whereas the plots from *cis* UFA-derived membranes had discontinuities. This is precisely the opposite of the pattern observed by Mavis and Vagelos (72).

#### Requirement for Lipids with Specific Physical Properties

**Requirement for membrane liquidity.** The isolation of UFA auxotrophs demonstrated that UFAs are required for normal membrane function. Since UFAs bestow liquidity on the membrane lipids, an absolute requirement for liquidity is indicated. Normally, *E. coli* lipid composition consists of phospholipids with one unsaturated and one saturated fatty acid (19, 98). *E. coli* cells containing such lipids have a membrane transition that extends from below 0 C to the growth temperature (108). Therefore, the membrane lipids of *E. coli* are ordinarily quite disordered. Is a high degree of disorder in the lipid phase necessary for cell growth? The cell appears to possess mechanisms for maintaining a high degree of membrane liquidity even during the incorporation of unnatural exogenous fatty acids. The amount of a given fatty acid incorporated by UFA auxotroph is inversely dependent on its fluidizing properties (26, 28, 100). Therefore, in the face of supplementation of UFA auxotrophs with very diverse fatty acids, the supplements are incorporated so as to assure the fluidity of the cell membrane at the growth temperature.

In this regard, Sinensky (106) has shown that *E. coli* exercises control of membrane lipid viscosity. By using a spin-labeled stearic acid, he measured, at different temperatures, the relaxation time in membranes and isolated lipids from cells grown at different temperatures. Viscosity was shown to be directly proportional to the relaxation time. He found that the variation in the viscosity of *E. coli* lipids measured at the growth temperature of the cells from which the lipids were derived is constant. However, the variation in viscosity of lipid extracts from cells grown at a single temperature varied by about 100-fold when assayed over the same temperature range. Similar results were obtained with membrane preparations. Therefore, *E. coli* seems to possess a mechanism (termed

"homeoviscous adaption") to produce membranes whose lipids have a constant fluidity despite the temperature of growth. This mechanism of this adaption is presumably an aspect of the temperature control of fatty acid composition (see below).

Physiological studies with UFA auxotrophs suggest distinct limits to the amount of fluidity required for cell integrity and growth. For instance, Overath et al. (81) found that *E. coli* will not grow on elaidate as the sole unsaturated fatty acid unless the growth temperature exceeds 37 C. Indeed, Esfahani et al. (28) have observed lysis of such cultures upon shift to lower temperatures. An upper limit to liquidity is indicated by the finding (81) that a linolenate-supplemented UFA auxotroph will not grow above 40 C. These results are readily interpreted in terms of lipid phase changes. However, the fact remains that the fatty acids incorporated are not natural components of *E. coli* (12), and hence the effects discussed above could be due to disturbances in other membrane processes. A definitive answer to the fluidity question therefore requires a method for limiting membrane fluidity while avoiding the use of unnatural fatty acids.

**Minimum UFA content.** What is the minimum amount of UFA required to support the growth of *E. coli*? Controlling the synthesis of UFA is one method of limiting membrane fluidity. Cronan and Gelmann (16) limited the synthesis of UFA by growing a *fabA* temperature-sensitive auxotroph (*fabA2*) at semipermissive temperatures. Such cultures grew more slowly than normal for long periods of time (several days) but could be restored to normal growth by addition of oleate. These cultures, therefore, approached the minimum UFA content consistent with growth at 34 C, the semipermissive temperature. This UFA level was 15 to 20% of the total phospholipid fatty acid, about one-third the normal content. The saturated fatty acid present was largely palmitic acid, and thus 80 to 85% of the lipids were dipalmitoyl lipids that would be largely in an ordered state at 34 C. This is consistent with DSC measurements (R. A. Mavis, personal communication) on envelopes from cells containing 26% UFA that have a transition beginning only above 35 C. Thus, it is evident that *E. coli* can grow despite much of the membrane lipid being in the ordered state. It should be noted that the fatty acid compositions of the inner and outer membranes of such UFA-limited cultures are very similar (C. Johnston and J. Cronan, unpublished data). Similar studies and conclusions were presented by Steim (108), who limited

UFA synthesis by addition of 3-DNAC, an inhibitor of UFA synthesis (see above). Although extensive data were not presented, the transition (as measured by DSC) was almost entirely above the growth temperature. Using the calibrations provided by Cronan and Gelmann (16) many subsequent workers have also found that 15 to 20% UFA is required for the growth of *E. coli* at 37 C. As would be expected from the phase properties of lipids, less UFA was required for growth at temperatures above 37 C (e.g., 11% UFA at 45 C) and more UFA for growth at lower temperatures (32% at 27 C). However, these later estimates by Davis and Silbert (21) may be slightly inaccurate because of the accumulation of large amounts of myristate in the phospholipids of such cultures. The physical properties of myristate may partially compensate for the lack of unsaturates.

If only 15 to 20% UFA is needed for growth, why is 50% UFA the composition of the native *E. coli* membrane? In nature, *E. coli* is exposed to sudden temperature decreases. Since the minimum UFA content is inversely dependent on temperature, 15 to 20% UFA would allow little fluidity at lower temperatures and would result in cell death. Therefore, it seems most likely that the "excess" fluidity is needed for environmental adaption. It should also be noted that synthesis of an unsaturated fatty acid requires only slightly more energy (that required to synthesize the needed enzymes) than does synthesis of a saturated fatty acid.

An illustration of the "excess" fluidity in the normal *E. coli* membrane is provided by the *Cvc<sup>-</sup>* mutant (34). This mutant is defective in the synthesis of *cis*-vacenate and thus, unlike wild-type cells, is unable to increase its content of UFA upon shift to lower temperatures (see below). However, despite this defect the strain grows normally at lower temperatures (34).

**Minimum saturated fatty acid content.** The question of fluidity can be reversed: Does *E. coli* require ordered membrane lipid for growth? The answer to this question appears to be affirmative, but the experiments cannot be interpreted as clearly as the UFA experiments discussed above. This caveat is necessary since saturated fatty acids are found in the lipid A component of the outer membrane as well as in phospholipid (19, 78). The isolation of auxotrophs requiring both a saturated fatty acid and a *cis* unsaturated fatty acid for growth and the action of cerulenin, an antibiotic that specifically engenders this dual requirement in wild-type cells, argues convincingly that saturated fatty acid is needed for growth. Using the *E. coli* fatty acid auxotrophs, Davis and Silbert

(21) have observed that decreasing saturated fatty acid content below 15% of the total caused a loss of the membrane barrier properties toward small molecules. However, lipid A synthesis is known to be required for growth (78), and thus this minimum saturated fatty acid requirement could be attributed to its scarcity in both lipid A and phospholipid.

**Significance of temperature-induced alteration in fatty acids.** The physical properties of the phospholipids of *E. coli* depend markedly on the growth temperature of the cells from which the lipids were isolated (38, 106). This change is due to an increased content of UFA (chiefly *cis*-vaccenate) in cultures grown at lower temperatures (see below). Sinensky (106) has reported that this compositional adaptation serves to maintain a constant membrane fluidity independent of the growth temperature. However, the properties of the Cvc<sup>-</sup> mutant indicate that this alteration is not of immediate vital importance to the cell. This strain is defective in the elongation of palmitoleate to *cis*-vaccenate having only 5 to 10% of the normal amount of *cis*-vaccenate. Due to this lesion, this strain is unable to increase its content of UFA after temperature decrease (34). Despite this defect, the strain grows normally upon temperature decrease. Similar conclusions were drawn by Shaw and Ingraham (94).

It seems paradoxical that a lesion in this regulatory process does not result in any observable growth defect. There are two possible explanations. First, it can be argued that the cell requires for growth at low temperature an increased *cis*-vaccenate content in some minor lipid subspecies. This minor component may not have been detected in the analysis of the total phospholipid fraction. However, rather detailed analysis of the Cvc<sup>-</sup> strain phospholipids from cultures shifted to 15°C for various periods of time (zero to eight generations) has not disclosed such a component (E. Tunaitis and J. Cronan, unpublished data). This result is in contrast to the reports of Kito and co-workers (1, 54, 55). These workers have reported different fatty acid compositions for each of the three phospholipid species of *E. coli* B (54) and that phosphatidylglycerol is selectively enriched in *cis*-vaccenic acid after temperature decrease (1, 55). The data presented in support of the conclusions of these workers are not compelling. The differences noted are small (<10% of the total fatty acid composition changes). Furthermore, the results seem largely due to experimental error since the data presented in the two papers on *E. coli* B do not agree well. For instance, Kito et al. (54) reported that the acyl groups of

the cardiolipin isolated from *E. coli* B grown at 40°C consist of 66% *cis*-vaccenic acid, whereas an apparently identical experiment by Aibara et al. (1) gave a figure of 36%. Similar experiments with *E. coli* K-12 also seem within the range of experimental error (55).

We therefore favor a second hypothesis that suggests that increased UFA content may have an evolutionary or other long-term selective advantage in nature. For instance, a 1% increase in growth rate would not be detected in the laboratory but would be a great advantage during the course of natural selection.

## REGULATION OF LIPID PHYSICAL PROPERTIES

### Possible Lipid Alterations Producing Altered Phase Transitions

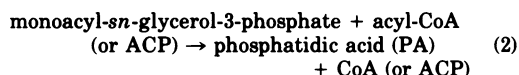
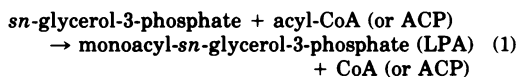
Model system studies indicate that several types of lipid changes could alter the phase properties of the *E. coli* membrane. For instance, an increase in the average chain length of the acyl chains or a decrease in the ratio of unsaturated to saturated moieties would raise the transition temperature. Replacement of phospholipids with normal acyl group composition with lipids containing two saturated or two unsaturated fatty acids would increase the number of ordered chains at elevated temperatures and might also result in phase separation. The increased heterogeneity of these lipids should also increase the breadth of the transition. It is also clear that a change in the distribution of polar head groups could change the transition (e.g., the phase behavior of dipalmitoyl phosphatidylserine differs greatly from that of the analogous ethanolamine derivative [see reference 57]). The interaction of various polar groups with divalent cations could also change the transition markedly (111, 117).

Experimental alteration of the polar groups of the *E. coli* phospholipids has not yet been studied. The recent isolation of a mutant in phosphatidylethanolamine synthesis that accumulates phosphatidylserine should permit such studies (43). Our discussion, therefore, will concentrate on the regulation of acyl chain length and of the degree of unsaturation.

### Sites of Control

**In vitro studies.** The enzymatic studies have focused mainly on the specificity of the acyl-transferases. However, some observations on the fatty acid synthetase and its component enzymes are relevant and will be discussed below.

(i) **Acyltransferase specificity.** Phosphatidic acid is a key intermediate in the formation of all the phospholipid species of *E. coli*. The synthesis of phosphatidic acid is believed to proceed by the following two acylation reactions:



(ACP is acyl carrier protein, LPA is lysophosphatidic acid, and PA is phosphatidic acid).

The acyltransferases that are bound to the inner membrane (5, 119) seem to consist of at least two enzymes. Incubation of a membrane preparation with a palmitoyl thioester and *sn*-glycerol-3-phosphate results primarily in the synthesis of 1-acyl-*sn*-glycerol-3-phosphate (86, 105, 116). In contrast, incubation with an unsaturated thioester results in the formation of the 2-isomer. The acyltransferases of *E. coli* are yet to be solubilized and purified (72). However, convincing though indirect evidence indicates that a single enzyme (or enzyme complex) is responsible for both specific acylations. Two different classes of acyltransferase mutants (4, 17) become defective in both enzymatic activities as the result of a single mutational event (14, 86; R. M. Bell, *J. Biol. Chem.*, in press). In addition, chemical modification results in the coordinate loss of both activities (86), and the two activities are similarly altered by alteration of the acyl composition of the membrane with which they are associated (72).

In vitro, the thioester moiety of the acyl donor can be either CoA or ACP. Although acyl-ACP is thought to be the normal acyl donor in vivo, this has not yet been proven (19). Acyl-ACP and acyl-CoA are transferred into phospholipid by the same enzyme (or enzyme complex) since the two classes of acyltransferase mutants have similarly altered enzyme activities with either substrate (T. K. Ray and J. E. Cronan, Jr., *J. Biol. Chem.*, in press).

The further acylation of the monoacyl compound to phosphatidic acid (reaction 2) seems complex at the present time. An enzyme activity capable of acylating 1-acyl-*sn*-glycerol-3-phosphate is present (44, 86, 105, 116; Bell, in press). A mutant in this enzyme was reported by Hechemy and Goldfine (44). This mutant, which also has a temperature-sensitive aldolase (which might have caused the in vivo phenotype), was lost during storage, thus precluding definitive study (H. Goldfine, personal com-

munication; 1102). Inactivation of this enzyme by heat (44, 86), maleation (86), or phospholipase C digestion (71) results in a biphasic inactivation curve that suggests two classes of enzymatic activity. Only one study on the acylation of 2-acyl-*sn*-glycerol-3-phosphate has been reported (77). Unfortunately, the atypical membrane preparation used by these workers makes the reported lack of activity for this substrate difficult to interpret (see below).

Most naturally occurring phosphoglycerides contain UFAs preferentially esterified at position 2 and saturated fatty acids at position 1 on the glycerol molecule (see reference 115 for a comprehensive review). The fatty acids of the *E. coli* phospholipids are asymmetrically distributed with palmitate esterified at position 1 and UFAs primarily esterified at position 2 of the glycerol backbone (19). The fatty acid compositions of the various phospholipid species are quite similar (19), as would be expected since all the phospholipids are derived from phosphatidic acid. At what level does positional specificity arise? A likely origin of the asymmetric distribution of fatty acids is in the esterification of *sn*-glycerol-3-phosphate to form phosphatidic acid. Results of van den Bosch and Vagelos (116), later confirmed by Ray et al. (86) and Sinensky (105) have shown that both the acylation of *sn*-glycerol-3-phosphate in reaction 1 and the acylation of 1-acyl-*sn*-glycerol-3-phosphate in reaction 2 proceed with great specificity. Incubation of an envelope fraction with *sn*-glycerol-3-phosphate and palmitoyl-CoA resulted in the formation of monoacyl-*sn*-glycerol-3-phosphate in which 92% of the palmitate was in the 1 position (86). In contrast, when the acyl donor was oleyl-CoA, palmitoleyl-CoA, or *cis*-vaccenyl-CoA, the fatty acid of the monoacyl-*sn*-glycerol-3-phosphate produced was mainly in the 2-position. Myristate was found to be distributed between both positions, with position 2 predominating. Similar specificity studies have been carried out in which 1-acyl-*sn*-glycerol-3-phosphate acyltransferase was tested with saturated and unsaturated thioesters. In this instance, acylation proceeded at much higher rates with unsaturated thioesters than with saturated thioesters (116). These in vitro reactions result in products (86, 105, 116) that are remarkably consistent with the fatty acid composition of the phospholipids found in the intact cells (19).

These acylation reactions are also thought to be one of the levels at which the temperature adaptation of the phospholipid composition is effected. Sinensky (105) has shown that the species of phosphatidic acid synthesized in vitro when the particulate enzyme preparation was

incubated at various temperatures with *sn*-glycerol-3-phosphate and a mixture of saturated and unsaturated acyl-CoA derivatives was quite consistent with the species of phospholipids found in cells grown at the same temperatures.

Recent data of Okuyama and Wakil (77) disturb this consistent scheme for positional specificity. These workers report the acylation of *sn*-glycerol-3-phosphate to be less specific than that observed by the earlier workers. In agreement with the previous results, palmityl group was incorporated specifically into position 1. However, unsaturated acyl chains were also found predominately in position 1, and only 20 to 30% of the total unsaturated chains were esterified to position 2.

These experiments were done by using procedures very similar to those used by the earlier workers except for the method of enzyme preparation (77). Okuyama and Wakil obtained their particulate membrane preparation after very vigorous sonic oscillation treatments. These treatments resulted in yields of membrane protein being only about 10 to 20% of the amount normally recovered. The specific activity for the acylation of *sn*-glycerol-3-phosphate was also quite low compared with the activities reported by other workers. These results suggest that Okuyama and Wakil (77) may have lost or destroyed a membrane component involved in the specific asymmetric acylation of *sn*-glycerol-3-phosphate. In addition, Okuyama and Wakil found that magnesium inhibited the acylation of 1-acyl-*sn*-glycerol-3-phosphate by their preparation, whereas all the other reports in the literature indicate that magnesium stimulates the reaction markedly (56, 73, 86, 116; Bell, in press; 62a). This result suggests further that the anomalous results of Okuyama and Wakil are due to their method of enzyme preparation. An exciting prospect raised by this work is that mixing various membrane fractions solubilized by incremental sonic treatments might localize asymmetric acylation activity in a discrete fraction and thus provide an assay for the factor conferring the positional specificity.

(ii) **Fatty acid synthetase.** The fatty acid synthetase of *E. coli* (usually assayed in a crude membrane-free supernatant of the whole cell) will synthesize fatty acids from malonyl-CoA and acetyl-CoA (7, 34, 52, 62, 85, 104). The absolute rate in vitro is very low compared with the cells from which the synthetase was derived (<1%) (7). The spectrum of fatty acid chain length synthesized by this system does not accurately reflect the in vivo conditions. *cis*-Vacenic acid is about 70 to 80% of the total fatty acid formed in vitro (7, 34, 52, 62, 85, 104). Only

small amounts of palmitoleic acid and saturated fatty acids are detected as end products. The saturated fatty acids, like the unsaturated acids, tend to be longer than those found in vivo (7, 34, 52, 62, 85, 104). This lack of fidelity of the synthetase to the in vivo system makes conclusions based on this system necessarily tenuous. The low overall activity might be due to inactivation of enzymes early in the pathway and might accentuate the terminal synthetic steps, thus resulting in the longer chain lengths and the predominance of unsaturates.

Greenspan and co-workers (37) studied the elongation specificity of purified  $\beta$ -ketoacyl-acyl carrier protein synthetase, the condensing enzyme of the fatty acid synthetase. These workers found the enzyme unable to elongate palmityl and *cis*-vaccenyl acyl carrier protein substrate although shorter chain lengths, both saturated and unsaturated, were readily elongated. These workers (37) therefore suggested that the specificity of the condensing enzyme was the primary mechanism determining the chain length of the fatty acyl chains of the *E. coli* phospholipids. However, recent studies (discussed below) indicate that this enzyme is not a primary determinate of chain length.

**In vivo studies.** Studies on UFA auxotrophs have given some in vivo information concerning the control of acyl group composition. The auxotrophs were found to acylate exogenous UFA preferentially at position 2 of the phospholipids, indicating that specific acylation was not dependent on UFA synthesis (98). Further studies showed, however, that the specificity of the acyltransferases was not absolute. Starvation of a UFA auxotroph results in the synthesis of phospholipids containing two saturated fatty acids (98). Therefore, specific acylation also depends on the supply of fatty acids (if the acyltransferase were completely specific an accumulation of monopalmityl *sn*-glycerol-3-phosphate would have been found). These results therefore suggest that control is shared by the acyltransferases and the fatty acid synthetase system.

(i) **Determination of fatty acid chain length.** We have found that *E. coli* accumulates free fatty acids during the absence of phospholipid synthesis (19a). After a blockage of phospholipid synthesis (by starvation of the appropriate auxotroph for glycerol), the synthesis of free fatty acid commences and proceeds at a steadily increasing rate until fatty acids are synthesized at the rate observed in glycerol-supplemented cultures (19a). The free fatty acid accumulated consists of an approximately equimolar mixture of saturated and unsaturated species. The fidelity of this system to normal

synthesis, therefore, seems quite good in this respect. The accumulated fatty acids are exceptionally long. Novel saturated acids of 20 and 22 carbons are found as well as a very large amount of stearic acid. The unsaturated fatty acids consist of *cis*-vaccenate and a third novel product, *cis*-13-eicosenoic acid. These results are similar to those found in gram-positive bacteria by Mindich (75).

The predominance of abnormally long chain lengths in the free fatty acid fraction indicates that a competition between fatty acid elongation and the transfer of acyl groups to *sn*-glycerol-3-phosphate determines the chain length of the acyl groups found in the lipids of *E. coli*. If acyltransfer is not permitted, the fatty acyl thioesters continue to be elongated and appear as abnormally long free fatty acids. However, it should be noted that the chain length of these free fatty acids is only about 30% longer than normal, indicating that some specificity as to the chain lengths synthesized resides in the fatty acid synthetase, perhaps in the  $\beta$ -ketoacyl acyl carrier protein synthetase (37).

(ii) **Regulation of the saturated-to-unsaturated fatty acid ratio.** The arguments given above strongly suggest that regulation of the ratio of saturated to unsaturated acyl chains found in the phospholipids does not reside solely in the acyltransferase. This was demonstrated by *in vivo* experiments in which the dosage of the *fabA* gene which codes for the enzyme  $\beta$ -hydroxydecanoyl thioester dehydrase was increased by genetic manipulation (13). The amount of this enzyme (which introduces the double bond of the unsaturated fatty acids) is doubled by the making the *fabA* gene diploid (13). This alteration causes a small increase in the UFA content of cells growing at constant temperature but an almost twofold increase in the rate of UFA synthesis in cells exposed to a sudden decrease in temperature (13). The decrease in temperature was designed to overcome temperature-dependent acyltransferase specificity (see the results of Sinensky [105] discussed below). These results show that the unsaturated-to-saturated ratio of the phospholipids of *E. coli* is partially determined by the levels of at least this fatty acid biosynthetic enzyme. The fact that cells growing at constant temperatures show no large increase in UFA content suggests the acyltransferase or some other factor also acts to determine the degree of unsaturation.

(iii) **Temperature control of fatty acid composition.** It was first noted by Marr and Ingraham (70) and later by many other workers (for review see reference 19) that *E. coli* adjusts

the fatty acid composition of its phospholipids in response to growth temperature. As the temperature of growth is lowered, the proportion of *cis*-vaccenic acid in the membrane lipids increases (19). The increase in *cis*-vaccenate content is not required for growth at the lower temperature (see above).

The mechanisms responsible for temperature-induced fatty acid alteration seem to operate at the levels of both phosphatidic acid synthesis and fatty acid synthesis. The *in vitro* studies of Sinensky (105) on the acyltransferases of *E. coli* indicate that these enzymes possess properties consistent with their having a role in the thermal regulation process. The relevance of these results rests on the *in vivo* experiments he also reported (105). Those experiments tested the effects of temperature on the incorporation of exogenously supplied saturated and unsaturated fatty acids into the phospholipids of *E. coli*. He found that the ratio of exogenous saturated to unsaturated fatty acids incorporated into phospholipid increased with increasing growth temperature. However, these results were somewhat compromised by his finding that the maximal ratio was observed at 30 C rather than at higher growth temperatures. Sinensky (105) also found much larger differences (fivefold) in this ratio between 10 C and 30 C cultures than are normally observed when phospholipid acyl groups are synthesized endogenously. These results were attributed by Sinensky to a dilution of the exogenously supplied unsaturated fatty acids by endogenous synthesis (105). Since this difficulty can now be overcome by use of cerulenin, the experiment of Sinensky has been repeated (Cronan, J. Biol. Chem., *in press*).

When cerulenin-treated cultures were grown with a mixture of [ $^3$ H]palmitate and [ $^{14}$ C]*cis*-vaccenate at various temperatures in the presence of cerulenin, at least 85% of the phospholipid fatty acyl moieties in these cultures were derived from these exogenously supplied fatty acids. These phospholipids were found to contain different ratios of the two acids. These ratios depended directly on the temperature of growth (Cronan, J. Biol. Chem., *in press*). The results were very similar to those expected if the acids had been synthesized endogenously and thus indicate a temperature-dependent change in acyltransferase specificity. Controls based on the  $\beta$ -oxidation of exogenous fatty acids showed that these results cannot be attributed to effects on fatty acid transport or activation.

However, even in the absence of these more rigorous *in vivo* studies, it was clear that acyl-

transferase specificity could not alone account for the increased *cis*-vaccenate content seen at low temperatures. The increased amount of *cis*-vaccenate must be provided by the fatty acid biosynthesis pathway. Introduction of the double bond of *cis*-vaccenate seems to be the rate-limiting step in the synthesis of this acid after temperature decrease (13). This is shown by the experiments using strains diploid for the *fabA* gene (discussed above). Therefore, some control resides at the branch point (or points) between saturated and unsaturated fatty acid synthesis.

Direct evidence for temperature control of acyl group composition at the level of fatty acid synthesis has come from experiments similar to those on chain length control discussed above. The ratio of saturated to unsaturated species in the free fatty acids that accumulate during an inhibition of phospholipid synthesis is very sensitive to temperature (Cronan, J. Biol. Chem., in press). The free fatty acid fraction from such cultures incubated at 15 C are 10-fold enriched in UFA compared with 43 C cultures.

In wild-type cells the elongation of palmitoleate to *cis*-vaccenate may also be temperature controlled. A reasonable control step may involve modulating the activity of the new  $\beta$ -ketoacyl acyl carrier protein synthetase recently reported by D'Angelo and co-workers (20a). Consistent with this possibility is the finding that the Cvc<sup>-</sup> strain may be deficient in this enzyme (P. R. Vagelos, personal communication).

### CONCLUSION

The lipids of the inner membrane of *E. coli* undergo a classical order-disorder phase transition as the temperature is increased. At this time, it seems most likely to us that lipid phase separations occur on a large scale in the *E. coli* membrane only when the lipids found normally in *E. coli* are rather homogenous in their properties (19), it follows that large-scale phase separations do not occur in the normal cell. To reconcile this view with the results of Linden and co-workers (66), we have suggested that the TEMPO spin label used by these investigators may not be measuring large-scale lipid phase separations in *E. coli* membranes.

The lipid phase properties of the membrane are of vital importance to *E. coli*. This is demonstrated by the finding that both fluid and nonfluid phospholipid molecules are required for growth. The synthesis of membranes containing less than one-third the normal amount of either fluid or nonfluid lipids results in cell death. However, within these limits, cell growth is quite insensitive to the ratio of fluid

to nonfluid lipid in the membrane. These results suggest that few specific interactions occur between the bulk lipid and other membrane components.

The function of the lactose transport system is directly dependent on the order-disorder state of the membrane lipids. This dependence could be due to lipid-associated events at either this transport system's substrate binding, translocation, or energy coupling steps. More detailed study of interactions with membrane lipid will require techniques allowing disruption and reassembly of the transport system. Other clues may come from interpretation of spin label results since the environments detected by the TEMPO label in some cases seem similar to those affecting lactose transport. It seems possible that the TEMPO label may be detecting exposure of hydrophobic protein regions.

Studies with membrane-bound enzymes suggest a considerable heterogeneity in the associations of membrane lipids with such enzymes. The rate-temperature profiles of some enzymes are quite sensitive to manipulation of the phospholipid acyl chains. However, no simple picture emerges. Some of the differences in activity occur at temperatures either above or below the disorder-order transaction and often no change is observed within the range of the transition. Furthermore, had a consistent pattern emerged, it would probably not have led to a clear structural scheme or functional interpretation. For instance, although many pure enzymes are known to show sharp discontinuities in Arrhenius plots (68), no useful structural information has been derived from this property (T. A. Steitz, personal communication).

*E. coli* normally controls the physical properties of its membrane by manipulating either endogenous or supplemental fatty acids to maintain a relatively constant state in response to temperature changes. Our understanding of how *E. coli* regulates the membrane lipid phase transition has been recently revised. Earlier results suggested that acyltransferase specificity could be solely responsible for regulation of the fatty acyl group composition. However, recent results demonstrating temperature control of fatty acid synthesis (Cronan, J. Biol. Chem., in press) and modification of the ratio of saturated to unsaturated acyl groups by manipulation of a fatty acid synthetic enzyme (13) indicate that regulation also resides at the level of fatty acid synthesis. In turn, the accumulation of abnormally long acyl chains in the absence of acyltransferase activity indicates that regulation at the level of fatty acid synthesis is

not sufficient to dictate the normal phospholipid acyl group composition (Cronan et al., 19a). The lipid phase transition, therefore, is jointly regulated at the fatty acid and the phospholipid synthetic levels. Considering the vital importance of lipid physical properties to the cell, the dual and overlapping nature of this control is not surprising. The molecular basis of this regulation is one of the more important unstudied areas in membrane function.

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